Nematode surface sterilization

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Updated date: Sep 2, 2021

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An abbreviated version of this protocol was published in Science Advances in Jul 2021

Root-knot nematode chemotaxis is positively regulated by l-galactose sidechains of mucilage carbohydrate rhamnogalacturonan-I

DOI: 10.1126/sciadv.abh4182

Detailed protocol

Protocol: (based on Nishiyama et al., 2015)
*all steps with nematodes uncovered are to be performed in a laminar flow hood

Transfer the suspension of harvested nematodes into autoclaved 10 ml glass conical test tubes with caps. Assume 2/3 of the nematodes will be lost during sterilization, and start with 3 times the number of sterilized nematodes that will be needed.

Centrifuge the nematode suspension at 1000 rpm for 1 min., discard the supernatant using autoclaved glass Pasteur pipettes.

Re-suspend the nematodes with 5 ml of sterilization solution per test tube, incubate in laminar flow hood in room temperature for 10 min.

Centrifuge nematodes at 1000 rpm for 1 min., discard the supernatant. Dispose the sterilization solution supernatant in accordance to mercury waste disposal guidelines.

Re-suspend the nematodes with 5 ml sterile ddH$_2$O per test tube to wash. Centrifuge at 1000 rpm for 1 min., discard the supernatant. Repeat for a total of 3 washes, dispose the washes as mercury wastes.

Re-suspend nematodes with 10 ml sterile ddH$_2$O per test tube, decant the nematode suspension into a sterile 100 ml beaker. Thaw a 1000 ppm streptomycin stock aliquot in room temperature. Add streptomycin to the nematode suspension at the final concentration of 1 ppm, mix gently. Cover the beaker with a piece of autoclaved aluminum foil.

Incubate the nematode suspension in 12°C overnight in darkness.

Decant the nematode suspension into new autoclaved 10 ml glass conical test tubes with caps.

Centrifuge nematodes at 1000 rpm for 1 min., discard the supernatant.

Re-suspend the nematodes with 5 ml sterile ddH$_2$O per test tube to wash. Centrifuge at 1000 rpm for 1 min., discard the supernatant. Repeat for a total of 3 washes.

Lay 3 sheets of autoclaved Kimwipe on top of each other then fold them in half (such that the stack consists of 6 sheets). Soak the Kimwipes in sterile ddH$_2$O and place them on the rim of a sterile 100 ml glass beaker, such that the Kimwipes forms an indentation of about 1 cm in the center of the beaker. Tuck the corners of the Kimwipes into the beaker using autoclaved forceps if necessary. Slowly fill the beaker with sterile ddH$_2$O until it reaches the bottom of the Kimwipes indentation.

Re-suspend the nematodes with 10 ml of sterile ddH$_2$O, and pour the nematode suspension on the Kimwipes. Continue to slowly pour sterile ddH$_2$O into the beaker until the beaker is completely filled with water below the Kimwipes. Ensure no air bubbles are trapped between/under the Kimwipes, use autoclaved forceps to dislodge the bubbles if necessary.

Loosely cover the Kimwipes/beaker set-up with a piece of autoclaved aluminum foil. Incubate the nematodes in laminar flow hood in room temperature for 2 hours, turn off the lights of the laminar flow hood if applicable. Healthy, motile nematodes will bore through the Kimwipes and sink to the bottom of the beaker.

Set up a sterilized filtration system using a filter membrane with pore size of 10 µm [we use Isopore PC 10µm filter, 47mm (Millipore)]. Ensure the membrane is thoroughly wetted in sterile ddH$_2$O beforehand.

Carefully remove the Kimwipes from the beaker using autoclaved forceps without disturbing the nematodes at the bottom of the beaker. Pour the contents of the beaker into the sterile filtration system and allow the contents to be filtered by gravity. Rinse the beaker and filter system with excess sterile ddH$_2$O if necessary.

Add 5 ml sterile ddH$_2$O into an autoclaved glass Petri dish slightly larger than the 10 µm filter. Dismantle the sterile filtration system, carefully transfer the 10 µm filter into the glass Petri dish using autoclaved forceps. Shake the Petri dish gently to dislodge the nematodes from the filter. Remove the filter with autoclaved forceps.

Use a sterile glass micropipette tip to take an aliquot of the nematode suspension, observe using dissecting microscope to estimate the nematode concentration and verify their viability.

Nematode suspensions may be stored in 12°C for short-term periods, though ideally they should be used as soon as possible after sterilization to minimize contamination.

*To minimize contamination, ensure all equipment and reagents that come in contact with nematodes are sterilized appropriately beforehand, all procedures are carried out in laminar flow hood, and the sterilized nematodes are used as soon as possible. Infection assays using nematodes sterilized using this protocol should be free of contamination for at least a few weeks.

Solutions:
Sterilization solution [0.002% (w/v) mercuric chloride, 0.002% (w/v) sodium azide, 0.001% Triton X-100, store in darkness].
Sterile ddH$_2$O.
1000 ppm streptomycin, divide into 1 ml aliquots and keep in -30°C.

1. Tsai, A. and Sawa, S. (2021). Nematode surface sterilization. Bio-protocol Preprint. bio-protocol.org/prep1360.
2. Tsai, A. Y., Iwamoto, Y., Tsumuraya, Y., Oota, M., Konishi, T., Ito, S., Kotake, T., Ishikawa, H. and Sawa, S.(2021). Root-knot nematode chemotaxis is positively regulated by l-galactose sidechains of mucilage carbohydrate rhamnogalacturonan-I . Science Advances 7(27). DOI: 10.1126/sciadv.abh4182

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